

A STUDY OF THE CHROMOSOMES IN TWO SPECIES OF BATS (CHIROPTERA)¹

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The Chiroptera is an order of mammals in which the chromosome survey has progressed less than in other orders and, despite their wide distribution all over the world, our cytological knowledge of them has remained incomplete. With reference to the evolution of mammals, the order Chiroptera occupies a very important position, since they are closely related to the Insectivora, which in turn are of interest in their relation to the Primates. In view of these facts a thorough exploration of the chromosomes of these mammals is highly desirable.

The literature pertaining to the chiropteran chromosomes is filled with discrepancies. Van der Stricht (1910), the first to concern himself with the chiropteran chromosomes, recorded the approximate number as 9-10 in the oocytes of *Vesperugo noctula*. Athias (1912) in an incomplete account of the chromosomes of *Rhinolophus hiposideros* and *Vesperugo serotinus* reported about 16 chromosomes as haploid in the oocytes of the former species and 15-24 in the latter. Jordan (1912) counted over 24 chromosomes in the diploid group of a male bat (the species name not given) and reported an XO chromosome mechanism. Working with a species of bat (the name unknown), Hance (1917) reported an approximate number of 40 for the male diploid complex. One is impressed from the above accounts that these earlier workers were handicapped by inadequate technique and any conclusive evidence cannot be expected from them. The only author who succeeded to some extent in surveying the morphology of chromosomes in this field is Painter (1925). He determined the diploid number of chromosomes in the embryonal cells of the horse bat, *Nyctinomus mexicanus*, to be 48, but presented no evidence relating to the maturation divisions.

The present paper deals with the chromosomes of the Okinawa fruit bat, *Pteropus dasymallus inopinatus* Kuroda, a member of the Megachiroptera, and those of the Japanese horse-shoe bat, *Rhinolophus ferrum-quinum nippon* Temminck, belonging to the Microchiroptera. In the former species, the material proved favorable for a thorough study of chromosomes throughout spermatogenesis, while in the latter form only the primary spermatocytes came under observation because of the unfavorable season for collecting the material. But in view of the unsatisfactory status of the chromosome survey in this order, the publication of even such a fragmentary account seems justified.

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The chromosomes of Pteropus dasymallus inopinatus Kuroda

Pteropus dasymallus inopinatus Kuroda, commonly known as the Okinawa fruit bat, is a member of the Pteropodidae of the Megachiroptera. It is distributed throughout the Riu-Kiu Islands. Testicular material obtained from two specimens caught in different localities was employed for study. One of them came to hand through the generosity of Dr. Y. Yamashina and was killed in September 1940. This animal came from Okinawa and had been reared for a few years in the Yamashina Ornithological Institute, Tokyo. The other specimen was secured by the author at Nago, Okinawa in April 1941. The testes were removed in living condition and fixed in Flemming's solution with no trace of acetic acid. Subjected to the usual paraffin method, the sections were stained with Heidenhain's iron-haematoxylin and light-green. The observations were chiefly carried out on the former material, which was fixed at a more favorable season of the year.

Careful counting of several adequate metaphase figures of the spermatogonia gave 38 as the diploid number in every case. As shown in Figures 1 and 2, the chromosome complement of this form is remarkable not only for its comparatively low number, but also for the fact that the majority of the chromosomes are J- or V-shaped. Close examination reveals that, except for a pair of elongated rod-shaped elements together with a Y chromosome of minute size, all the other elements are provided with atelomitic fibre attachments. The latter are submedian or subterminal in location. The autosomes vary in size, ranging from enormous J- or V-shaped elements to small, slightly curved bodies. In the equatorial plate, the latter lie with the bend directed towards the centre of the plate and with their long axes stretching across the radius of the equatorial plate.

The two elongated chromosomes, in turn, are in striking contrast to the others due to their remarkable rod-shape. They form a homologous pair, identical in length, and seem to be of telomitic nature, since they have tapering ends at their inner extremities where the spindle fibres attach. They correspond in length to one arm of the largest V-shaped chromosome.

The even number of chromosomes herein established naturally suggests the occurrence of an XY-pair of sex chromosomes, as is the general rule in the mammals so far reported. On account of its size and shape, the Y chromosome is readily distinguishable from the others. The identification of the X element, however, presents some difficulty. It is probable that the X is represented by one of the atelomitic chromosomes. To judge from the morphology of the sex chromosome pair as disclosed in the first meiotic division, the X element seems to be represented by one of the submedian chromosomes, fairly large in size and having extremely dissimilar arms.

The chromosomes found in the primary spermatocyte metaphase are observable with extreme clearness due to the reduced number of elements. The chromosome count at this stage is therefore made with certainty and thus it gives final confirma-

tion of the diploid number obtained in the spermatogonia. Every metaphase plate under observation shows consistently 19 chromosomes, having various shapes and sizes (Figs. 3 to 5). The chromosomes are all bivalent in structure. The haploid complex consists of 18 autosomal bivalents with ordinary structure and an XY complex of heteromorphic nature, composed of the large J-shaped X and the small Y.² Corresponding to the morphology of the spermatogonial chromosomes, the autosomal bivalents are variable in size and the majority of them appear as atelomitic in their fibre attachment. Three or four of these bivalents are relatively small in size, in striking contrast to the remaining ones. Among the larger bivalents the size differences are slight and they form a closely graded series.

The XY-bivalent is readily distinguishable from the autosomal bivalents because of its unusual asymmetrical configuration, and by its relatively peripheral position in the metaphase plate (XY in Figs. 3 to 5). It consists of the J-shaped X element of large size and the very minute Y element. The latter is attached to the extremity of the long arm of the X. At metaphase, the X is placed so that its longer arm with the Y at its extremity is vertical to the equatorial plate, while the shorter arm lies in most cases parallel to the equatorial plate. Sometimes the longer arm of the X acquires two constrictions due probably to the elongation, giving as a result a somewhat undulating appearance (Figs. 6 and 7). To judge from the structural configuration, the spindle fibres of the X attach to the point where the long and short arms join, while in the Y they apparently attach to its free end. The whole configuration displayed by the XY bivalent, as outlined in the above description, is clearly displayed in the profile view of the metaphase plate of the primary spermatocyte, as given in Figures 6 to 8.

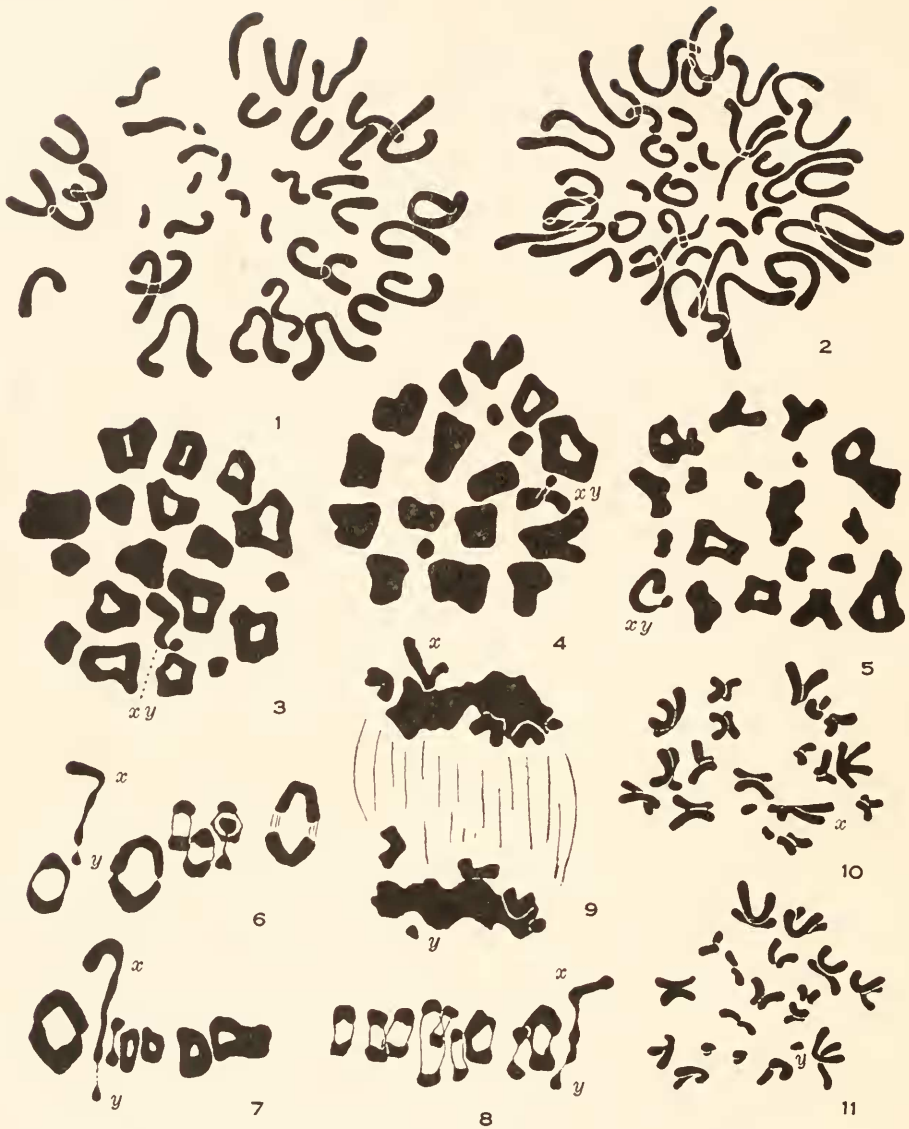
At the commencement of anaphase of the first division, the separation of autosomes takes place synchronously and there is usually no element which lags or precedes the others (Fig. 8). During the division of the autosomal elements, the X also disjoins from the Y and they migrate to opposite poles. The anaphasic picture is clearly seen in Figure 9.

There are discernible two kinds of secondary spermatocytes, though each has an equal number of chromosomes, resulting from the reductional segregation of the X and Y in the first division. The one consists of 18 autosomal elements and the X chromosome (Fig. 10), and the other contains a corresponding set of autosomal elements and the minute Y (Fig. 11).

*The chromosomes of *Rhinolophus ferrum-equinum nippon* Temminck*

The Japanese horse-shoe bat, *Rhinolophus ferrum-equinum nippon* Temminck, is a common species of the Rhinolophidae (the Microchiroptera) and known throughout Hokkaido, Hondo, Shikoku and Kyusyu. The testes of a male specimen which was obtained at Nagasaki in October 1937 were fixed with Flemming's solution without acetic acid by the late Dr. D. Nakamura. After imbedding in paraffin the material was sent to the author. Staining was done with Heidenhain's iron-haematoxylin and light-green.

² It will be understood that since no female material was available, the specific identification of the X and Y chromosomes is tentative. However, in view of the general agreement in the configuration of the sex chromosomes of other mammals, there would appear to be small doubt about its correctness.



All drawings at a magnification of $3200\times$ (reduced from $6000\times$)

FIGURES 1-11. Chromosomes of *Pteropus dasymallus inopinatus*. 1-2, Spermatogonial metaphases, showing 38 chromosomes. 3-5, primary spermatocyte metaphases, showing 19 bivalents. 6-8, side views of the first division metaphases, showing the XY complex. 9, side view of the first division anaphase, showing the segregation of the X and Y elements. 10, secondary spermatocyte metaphase, showing 19 dyads. X-class. 11, the same, Y-class.

The material proved to be insufficient for a complete study of the spermatogenesis, because of the unfavorable season of collection; consequently only fragmentary notes concerning the primary spermatocyte chromosomes can be given here.

The haploid number of chromosomes was determined in the primary spermatocyte. The author was surprised at finding a higher number of chromosomes in this species than that encountered in the previous form. Counts in several adequate plates established that there are 29 distinct chromosomes having bivalent nature. As seen in Figures 12 to 15, the chromosomes vary considerably in their size and shape. The configuration of some of the larger bivalents is strongly suggestive of subterminal or submedian fibre attachments. To the author's view, a few of the medium sized ones seem also to be of atelomitic nature. Four or five bivalents are extremely small in size. Outstanding in the haploid complement is a heteromorphic bivalent which is composed of a long rod-shaped element and a minute granular one, connected end to end with each other. Taking all characteristic conditions into con-



All drawings at a magnification of $3200\times$ (reduced from $6000\times$)

FIGURES 12-18. Chromosomes of *Rhinolophus ferrum-equinum nippon*. 12-15, primary spermatocyte metaphases, showing 29 bivalents. 16-18, side views of the first division metaphases, showing the XY complex.

sideration, it is concluded that this particular chromosome configuration is the XY-bivalent, the larger component being the X and the smaller the Y (XY in Figs. 12 to 15). The XY-bivalent usually takes a peripheral position in the metaphase arrangement.

It is in the side view of the metaphase spindle that the configuration and structure of the XY-bivalent are most clearly observed (Figs. 16 to 18). Morphologically considered, the X seems to be provided with a telomitic fibre attachment. The Y element is more or less spherical in outline and seems to be slightly smaller than the smallest autosome. The two chromosomes are connected to each other, end to end, by a fine fibre and are placed nearly perpendicularly to the equatorial plate in a linear configuration. The mode of conjugation as seen in the X and the Y is fairly comparable to that found in the mice as reported by the author (Makino, 1941).

On the basis of the above results, the following statement is possible: the diploid number of chromosomes in this species would appear to be 58, comprising both atelomitic and telomitic elements of varying size and shape. The sex chromosomes are of the usual XY type, the X having an elongated rod-shape and the Y a minute dot-like one.

REMARKS

It seems to the author that among the earlier works on chiropteran chromosomes the only account sufficiently accurate to warrant a more detailed discussion is that of Painter (1925) on the house bat, *Nyctinomus mexicanus*, a species of the Molossidae. Painter (1925) made the chromosome count in the anion of young embryos and established 48 chromosomes as the diploid number of this species. Of these, at least six pairs of chromosomes have a distinct V-shape (Painter, 1925, Plate I). In comparison with the present material, it is thus evident that the number of chromosomes of *Nyctinomus* is smaller than that of *Rhinolophus ferrum-equinum nippon*, in which 29 haploid chromosomes were clearly counted. However, no precise comparison is possible with the data at hand.

The present communication constitutes the first report on the chromosomes of one of the Macrochiroptera. As reported in the foregoing pages, the diploid number of chromosomes of the Okinawa fruit bat, *Pteropus dasymallus inopinatus*, is 38. Referring to the literature on mammalian chromosomes (see the list newly compiled by Makino, 1947), it is apparent that the chromosome number here established for this species is rather low in comparison with that of other eutherian mammals, so far as the recorded cases are concerned. Furthermore, specially remarkable is the fact that the karyotype of this form is probably archaic among the eutherian species so far studied, since, excepting one pair of long rod-shaped autosomes and the Y chromosome, the chromosomes are all provided with an atelomitic fibre attachment, the majority of them being V- or J-shaped and of large size.

No accurate and clear-cut evidence has been demonstrated for the sex chromosome mechanism of the Chiroptera in any of the previous investigations. Painter (1925) working with *Nyctinomus*, was unable to observe the sex chromosome of this species in the maturation division. He stated that the constancy of the chromosome number in different embryos together with the fact that in some embryos a pair of unequal sized elements is observed, would seem to indicate that we have in this form the XY type of sex chromosome. The evidence presented in the present study shows that the XY mechanism of sex determination occurs in both of the species under study. In both the X and the Y separate in the first division, and pass to opposite poles.

Painter (1925) counted the chromosome number in 10 species of eutherian mammals covering 7 different orders, and emphasized that 48 chromosomes occur in the most primitive eutherian order of Insectivora, and in such divergent orders as the Primates and Chiroptera. He deduced from this evidence that the chromosome number of 48 seems to be the typical and is probably the basal number for the mammals.

During recent years, the present author has engaged in a comparative study of mammalian chromosomes, endeavoring to make as broad a survey of Eutheria as possible. At present his investigations have extended to 42 species which repre-

sent 1 species of the Insectivora, 2 species of the Chiroptera, 22 species of the Rodentia, 4 species of the Carnivora, 8 species of the Artiodactyla, 2 species of the Perissodactyla, 1 species of the Cetacea and 2 species of the Primates. Some of the results have already been reported (Makino, 1942, 1943 a, b, c, 1944 a, b, c, 1946, 1947 a, b). The results of these studies demonstrate that there is a striking diversity in the number of chromosomes of the species studied, ranging from 30 in the Vole, *Microtus kikuchi* to 78 in the dog, *Canis familiaris*, and further that the chromosome number of 48 was proved to exist in only four out of 42 species studied—to wit—*Buballus buffelus* of the Artiodactyla, and *Apodemus agrarius*, *A. semotus* and *Lepus gichigannus ainu* of the Rodentia. Thus the newly obtained data clearly point to the fact that the occurrence of 48 chromosomes is relatively rare in mammals, and can no longer be regarded as the basal number. It is evident that our knowledge of the chromosomes of mammals is still too scanty to justify any generalization. To discover the basal number and to establish a phylogenetical relationship on the basis of cytology is a task which will require the accumulation of accurate data in an immense number of closely related forms, before one can attempt to formulate any rule of general application.

SUMMARY

The chromosomes of *Pteropus dasymallus inopinatus*, a species of the Macrochiroptera, were investigated in male germ cells. This species was found to possess the diploid number of 38 in spermatogonia and the haploid number of 19 in both of the primary and secondary spermatocytes. The karyotype of this form is noticeable in having an archaic constitution in comparison with that of other forms of mammals. Excepting a pair of elongated rod-shaped elements and a very minute Y chromosome, the remaining members of the diploid complex are all provided with atelomitic fibre attachment; the majority are J- or V-shaped. The sex chromosome mechanism was shown to be of the usual XY type. The X is represented by one of the large J-shaped elements, consisting of two arms of very unequal length, while the Y is a minute dot, the smallest of all. At meiosis the Y is found conjugated with the X at the free end of its longer arm. In the first division the X and the Y pass to opposite poles. There result two kinds of secondary spermatocytes, one with an X and the other with the Y.

Observations upon the primary spermatocyte chromosomes reveal that *Rhinolophus ferrum-equinum nippon* possesses a haploid number of 29. From this haploid number it may be assumed that the diploid number of this species is 58. Judging from the configuration of the bivalents, this complement comprises both telomitic and atelomitic members. A heteromorphic XY bivalent is very distinct in the haploid group. It consists of a larger rod-shaped component, the X, and a minute dot-like one, the Y, connected end to end. They segregate in the first division.

LITERATURE CONSULTED

- ATHIAS, M., 1912. Sur les divisions de maturation de l'oeuf des mammifères. *Arch. Inst. Bact. Cam. Pest. Lisbonne*, 3.
HANCE, R. T., 1917. The fixation of mammalian chromosomes. *Anat. Rec.*, 12.
JORDAN, H. E., 1912. Notes on the spermatogenesis of the bat. *Anat. Anz.*, 40.

- MAKINO, S., 1941. Studies on the murine chromosomes. I. Cytological investigations of mice, included in the genus *Mus*. *Jour. Fac. Sci. Hokkaido Univ. Ser. VI. Zool.*, 7.
- MAKINO, S., 1942. Studies on the murine chromosomes. II. Morphological comparison of chromosomes between the wild form and the domesticated variety of *Rattus norvegicus* Berkenhaut. *Ibid.*, 8.
- MAKINO, S., 1943a. Studies on the murine chromosomes. III. A comparative study of chromosomes in five species of *Rattus*. *Ibid.*, 9.
- MAKINO, S., 1944a. Studies on the murine chromosomes. IV. The karyotypes of the mole-rat and the harvest-mouse. *Cytologia*, 13.
- MAKINO, S., 1943b. Chromosome studies in domestic mammals. I. The chromosomes of the horse (*Equus caballus*). *Cytologia*, 13.
- MAKINO, S., 1943c. Chromosome studies in domestic mammals. II. The chromosome complexes of goat (*Capra hircus*) and sheep (*Ovis aries*) and their relationship. *Ibid.*, 13.
- MAKINO, S., 1944b. Chromosome studies in domestic mammals. III. The chromosome complex of the pig (*Sus scrofa*). *Ibid.*, 13.
- MAKINO, S., 1944c. Chromosome studies in domestic mammals. IV. Karyotypes of domestic cattle, zebu and domestic water-buffalo. *Ibid.*, 13.
- MAKINO, S., 1947a. Chromosome studies in domestic mammals. V. Notes on the chromosomes of four species of small mammals. *Jour. Fac. Sci. Hokkaido Univ. Ser. VI. Zool.*, 9.
- MAKINO, S., 1944. A contribution to the study of the chromosomes of domestic mammals (in Japanese). *Zool. Mag.* (Tokyo), 56.
- MAKINO, S., 1946. On the karyological relationship of the domestic swine and the Japanese wild boar (in Japanese). *La Kromosomo*, 1.
- MAKINO, S., 1947b. The chromosomes of the Dall's porpoise *Phocoenoides dallii* (True), with remarks on the phylogenetical relations of the Cetacea. (In press.)
- MAKINO, S., 1947c. A revised list of the chromosome numbers in animals. Tokyo (in press).
- PAINTER, TH. S., 1925. A comparative study of the chromosomes of mammals. *Amer. Nat.*, 59.
- VAN DER STRICHT, O., 1910. La structure de l'oeuf des mammifères. *Mem. Acad. Roy. Belg., Ser. 2*, 2.